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☐ **Brief Communication** ☐

A role of carboxy-terminal region of *Toxoplasma gondii*-heat shock protein 70 in enhancement of *T. gondii* infection in mice

Hye-Seong MUN, Kazumi NOROSE, Fumie AOSAI, Mei CHEN and Akihiko YANO*

Department of Parasitology, Chiba University School of Medicine, Chiba 260-8670, Japan

Abstract: We investigated the role of recombinant *Toxoplasma gondii* heat shock protein (r*T.g*.HSP) 70-full length, r*T.g*.HSP70-NH2-terminal region, or r*T.g*.HSP70-carboxy-terminal region in prophylactic immunity in C57BL/6 mice perorally infected with Fukaya cysts of *T. gondii*. At 3, 4, 5, and 6 weeks after infection, the number of *T. gondii* in the brain tissue of each mouse was measured by quantitative competitive-polymerase chain reaction (QC-PCR) targeting the surface antigen (SAG) 1 gene. Immunization with r*T.g*.HSP70-full length or r*T.g*.HSP70-carboxy-terminal region increased the number of *T. gondii* in the brain tissue after *T. gondii* infection, whereas immunization with r*T.g*.HSP70-NH_2-terminal region did not. These results suggest that *T.g*.HSP70-carboxy-terminal region as well as *T.g*.HSP70-full length may induce deleterious effects on the protective immunity of mice infected with a cyst-forming *T. gondii* strain, Fukaya.

Key words: *Toxoplasma gondii*, heat shock protein 70, heat shock protein 30, SAG1, QC-PCR

Heat shock protein (HSP) 70 apparently stabilizes non-native polypeptides through the binding of hydrophobic peptide segments, which are exposed during protein synthesis, protein translocation, and protein degradation (Demand et al., 1998). Functional and structural studies of HSP70 have suggested that the molecule contains two major domains: a highly conserved NH_2-terminal region, which contains the ATPase activity, and the more divergent carboxy-terminal region, which is thought to be important in substrate recognition (Demand et al., 1998).

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* Corresponding author (e-mail: yano@med.m.chiba-u.ac.jp)

An HSP70 has been shown to be a major immunogen in infections caused by a number of pathogens (Hedstrom et al., 1988; Behr et al., 1992; Wallace et al., 1992; Rico et al., 1998; Mun et al., 1999). It has been proposed that parasite-derived HSP70 may have potential as an antigen for vaccines (Behr et al., 1992; Yang et al., 1997; Rico et al., 1998). Behr et al. (1992) reported that *Plasmodium falciparum* HSP70 induced interferon-gamma (IFN-γ) production by patient's peripheral blood lymphocytes (PBL), and Rico et al. (1998) described that *Leishmania infantum* HSP70 induced IFN-γ production of lymph node cells from immunized mice. We have reported the existence of $CD4^+$ helper T lymphocytes and $CD4^+CD8^-$ and $CD4^-CD8^+$ cytotoxic T lymphocytes in peripheral blood lymphocytes of patients with toxoplasmosis (Yang et al., 1995) and demonstrated that human heat shock cognate protein 71 (HSC71) has an important role in antigen processing and

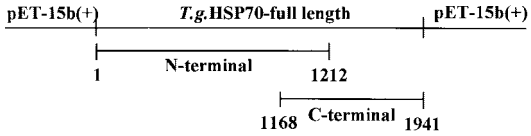

Fig. 1. Constructs of plasmids for recombinant protein product for *T.g.*HSP70-full length, *T.g.*HSP70-NH_2-terminal region, and *T.g.*HSP70-carboxy-terminal region. The cDNA of *T.g.*HSP70-full length (1941 bp; from 1 to 1941), *T.g.*HSP70-NH_2-terminal region (1212 bp; from 1 to 1212), and *T.g.*HSP70-carboxy-terminal region (774 bp; from 1168 to 1941) was cloned into the pET-15b (Novagen, Madison, USA) plasmid.

antigen presentation by *Toxoplasma gondii*-infected melanoma cells to a $CD4^+CD8^-$ cytotoxic T lymphocytes line specific for *T. gondii*-infected melanoma cells (Yang et al., 1997). We recently reported that the immunization of recombinant *T. gondii* (r*T.g.*) HSP70-full length inhibited host protective immunity in *T. gondii*-infected mice (Mun et al., 1999).

In order to localize the region of *T.g.*HSP70 involved in deterioration of *T. gondii* infection, we utilized *T.g.*HSP70-full length, *T.g.*HSP70-NH_2-terminal region, and *T.g.*HSP70-carboxy-terminal region. The cloning and expression of r*T.g.*HSP70-full length, r*T.g.*HSP70-NH_2-terminal region (1212 bp; from 1 to 1212), r*T.g.*HSP70-carboxy-terminal region (774 bp; from 1168 to 1941), and r*T.g.*HSP30/bag1, a bradyzoite-specific molecule, were done as described previously (Fig. 1) (Yano et al., 1998; Mun et al., 1999). Age/sex-matched C57BL/6 mice were immunized by intraperitoneal injection of 100 μg of r*T.g.*Hsp70-full length, r*T.g.*HSP70-NH_2-terminal region, r*T.g.*HSP70-carboxy-terminal region, or r*T.g.*HSP30/bag1 that had been suspended in 100 μl of phosphate-buffered saline (PBS) and emulsified with 100 μl of complete Freund's adjuvant (Hoechst, La Jolla, CA, USA). Control groups were injected with an emulsified mixture of 100 μl of PBS combined with 100 μl complete Freund's adjuvant. Ten days after immunization, the mice were infected with five cysts of the Fukaya strain of *T. gondii* via the oral route. The infected mice were sacrificed at 3, 4, 5, and 6 weeks after infection and the number of *T. gondii* was then measured by quantitative competitive-polymerase chain reaction (QC-PCR) targeting the surface antigen (SAG) 1 gene, as reported previously (Luo et al., 1997). In brief, genomic DNA (1 μg) extracted from the brain tissue of each mouse was coamplified with a constant amount of the competitor DNA using a set of SAG1-specific primers. The amplified products were separated by agarose gel electrophoresis and stained with ethidium bromide. The ratio of the staining intensities of the amplified target and competitor sequences were determined using an IPLab Gel Densitometer (Signal Analytical Corp., Vienna, VA, USA). By comparing the ratio obtained to a standard curve, the *T. gondii* number in the brain was estimated.

The number of *T. gondii* in the brain tissues of mice immunized with r*T.g.*HSP70-full length or r*T.g.*HSP70-carboxy-terminal region was markedly greater 4 to 6 weeks after the challenge infection with Fukaya cysts than that in the brains of control mice (Fig. 2). Interestingly, the number of *T. gondii* in the brain tissues of mice immunized with r*T.g.*HSP70-carboxy-terminal region was enhanced compared to that of mice immunized

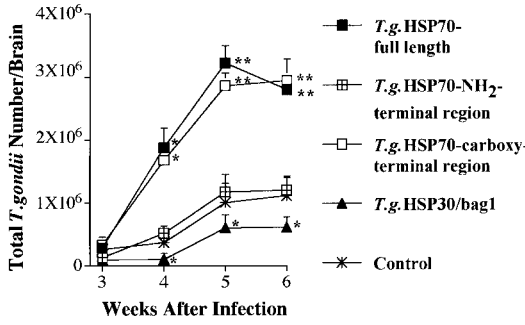

Fig. 2. Effects of immunization with r*T.g.*HSP70-full length, r*T.g.*HSP70-NH_2-terminal region, r*T.g.*HSP70-carboxy-terminal region, or r*T.g.*HSP30/bag1 on C57BL/6 mice infected perorally with the Fukaya strain of *Toxoplasma gondii*. At 3, 4, 5, and 6 weeks after infection, the infected mice were sacrificed and the number of *T. gondii* was then measured by QC-PCR targeting the SAG1 gene. The significance of differences between groups was determined by Student's *t*-test. $P<0.05$ was taken as significant.
* $P<0.05$ compared with control mice.
** $P<0.005$ compared with control mice.

with r*T.g.*HSP70-full length. In contrast, the number of *T. gondii* in the brain tissues of mice immunized with r*T.g.*HSP70-NH_2-terminal region was not significantly increased compared with that in control mice. Additionally, when mice were immunized with r*T.g.*HSP30/bag1, the number of *T. gondii* in the brain tissues of C57BL/6 mice was lower than that in control mice 4 to 6 weeks after the challenge infection (Fig. 2).

The antigenicities of these recombinant proteins in antibody formation were examined (Fig. 3). Formation of IgG antibodies against *T.g.*HSP70-full length, *T.g.*HSP70-NH_2-terminal region, *T.g.*HSP70-carboxy-terminal region, and *T.g.*HSP30/bag1 was examined in *T. gondii*-infected mice pre-immunized with or without the recombinant antigens. The sera were collected from each mouse and tested by ELISA (Mun et al., 1999).

Higher levels of IgG antibodies to *T.g.*HSP70-NH_2-terminal region and *T.g.*HSP30/bag1 as well as *T.g.*HSP70-full length and *T.g.*HSP70-carboxy-terminal region were observed in *T. gondii*-infected mice pretreated with recombinant antigens than in *T. gondii*-infected mice without pretreatment (Fig. 3). The data indicate that the immunogenicity of *T.g.*HSP70-NH_2-terminal region (and *T.g.*HSP30/bag1) in IgG antibody formation was equivalent to those of *T.g.*HSP70-full length and *T.g.*HSP70-carboxy-terminal region, and suggest that antibodies to *T.g.*HSP70 recombinant proteins were not involved in *T.g.*HSP70 mediated-enhancement of the number of *T. gondii* in the brain.

The deleterious effects induced by immunization with r*T.g.*HSP70-full length or r*T.g.*HSP70-carboxy-terminal region with respect to the mortality of infected hosts may be due to several reasons. The first possibility is the activation of immunosuppression by down-regulating nitric oxide release of macrophages. The second possibility is that *T.g.*HSP70-full length and *T.g.*HSP70-carboxy-terminal region may function as a source of autoimmunity. In our hands, preliminary data suggest these two possible mechanisms.

We demonstrated here that *T.g.*HSP70-NH_2-terminal region did not inhibit the host protective immunity (Fig. 2) as described

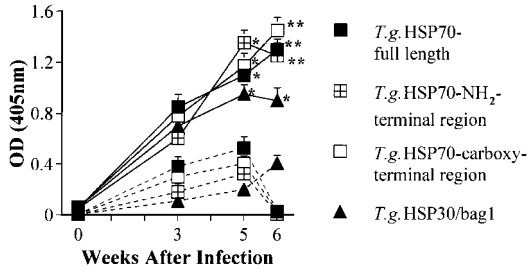

Fig. 3. IgG antibody production against *T.g.*HSP70-full length, *T.g.*HSP70-NH_2-terminal region, *T.g.*HSP70-carboxy-terminal region, and *T.g.*HSP30/bag1 in *Toxoplasma gondii*-infected mice pre-immunized with or without the recombinant antigens. The sera were collected from each mouse and analyzed by ELISA. The solid line represents mice pre-immunized with recombinant antigens and then infected with *T. gondii* cysts. The dotted line represents without pre-immunization of the recombinant antigens. ELISA titers of *T.g.*HSP70-full length, *T.g.*HSP70-NH_2-terminal region, *T.g.*HSP70-carboxy-terminal region, and *T.g.*HSP30/bag1 of control (non-infected, non-immunized) mouse sera were 0.045, 0.04, 0.05, and 0.04 respectively. The significance of differences between groups was determined by Student's *t*-test. $P<0.05$ was taken as significant.
* $P<0.05$ compared with non-immunized mice.
** $P<0.005$ compared with non-immunized mice.

above. These data indicate that the ATPase activity of *T.g.*HSP70-full length, which is critical for its role in protein refolding, is dispensable for *T.g.*HSP70-full length-mediated inhibition of the host protective immunity. Furthermore, since *T.g.*HSP70-carboxy-terminal region induces the deleterious effects on protective immunity as well as *T.g.*HSP70-full length (Fig. 2), *T.g.*HSP70-carboxy-terminal region may play a crucial role in *T. gondii* infection.

Wallace et al. (1992) reported that in visceral leishmaniasis infections, the carboxy-terminal region of *Leishmania donovani* HSP70 is a major target of the humoral immune response. Similarly, Hedstrom et al. (1988) reported that the immunodominant region of schistosome HSP70 is also located in the carboxy-terminal region, and the responses against *S. mansoni* and *S. japonicum* have been reported to be immunologically distinct and non-cross-reactive, although the epitopes involved were

identified. James et al. (1997) also demonstrated that the carboxy-terminal region is a potential source of functional differences because the carboxy-terminal regions of the Ssa1 and Ssb1 HSP70 families of *Saccharomyces cerevisiae* are only 14 % identical.

Experimental murine toxoplasmosis provides a suitable tool for evaluating immune reactions to *T. gondii* because it closely mimics human disease (Choi et al., 1995; Chai et al., 1997; Park and Nam, 1999). Our present data suggest that the immunization with *T.g.*HSP70-carboxy-terminal region induces the deleterious effects on protective immunity in mice infected with *T. gondii*. Further studies are currently underway to analyze the mechanisms of *T.g.*HSP70-carboxy-terminal region-induced deterioration of toxoplasmosis in the brains of *T. gondii*-infected mice.

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